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Inflorescences of *Phyllonoma* (Phyllonomaceae, Aquifoliales): Anatomical Observations

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Phyllonoma, the only genus of Phyllonomaceae (Aquifoliales), consists of four Central American species that have characteristic epiphyllous inflorescences. It has been long uncertain whether the inflorescences are cymose or racemose. The latest literature reports suggest that three species, *P. laticuspis*, *P. tenuidens* and *P. weberbaueri* (with small reduced inflorescences), have racemes, while *P. ruscifolia* (with large inflorescences) has irregular monochasial cymes. Anatomical observations of the inflorescence of *P. tenuidens* using serial transverse microtome sections show that it is not a raceme but a monochasial inflorescence, more strictly a scorpioid cyme. Results of the present study, together with earlier observations in other species, suggest that the entire genus *Phyllonoma* has monochasial cymes. Recaulescence by means of the adnation of a subtending bract (prophyll) to a higher order axis for some distance, resulting in the metatopic displacement of an axillary bud, is prevalent in the epiphyllous inflorescence.

Key words: epiphyllous inflorescence, *Phyllonoma*, Phyllonomaceae, *Phyllonoma tenuidens*

Phyllonoma Willd. ex Schult., the only genus of Phyllonomaceae (Aquifoliales), comprises four species of shrubs or trees endemic to Central America (Mori & Kallunki 1977). The species have epiphyllous inflorescences consisting of small flowers as in *Helwingia* Willd., the sister group of Phyllonomaceae. Recently I investigated the floral morphology and structure of *Phyllonoma* on the basis of flowers of *P. tenuidens* Pittier (Tobe 2013). The inflorescence structure of the species of *Phyllonoma* has remained uncertain, particularly with respect to whether or not the inflorescence branches. If branching takes place exclusively from the axils of the prophylls, the inflorescence is cymose. If not, it is racemose. Rusby (1905, p. 191) first described *Phyllonoma* as having a cymose inflorescence on the basis of *P. laticuspis* (Turcz.) Engl. Hutchinson (1967, p. 32) reported, however, that in *Phyllonoma* the inflorescence is “shortly racemose.” Stork (1956, p. 340) states that in *P. ruscifolia* Willd. ex Schult. the inflorescence is a cyme with several to 15 flowers. Dickinson & Sattler (1974, p. 3) commented on the inflorescence of *P. ruscifolia* (“*P. integerrima* Loes.”) that, “although Stork (1956) reports the inflorescence as cymose, the material examined exhibited a great deal of variation in the extent of development and branching within the mature inflorescence and infructescence.”

In a taxonomic revision of *Phyllonoma*, Mori & Kallunki (1977, p. 73) examined inflorescences of all four species, stating that they “are difficult to classify to type.” Based on their analyses, the inflorescences of *P. ruscifolia* differed from those of the other three species, *P. tenuidens*, *P. laticuspi*s, and *P. weberbaueri* Engl. In *P. ruscifolia* the inflorescence is usually branched, sometimes diffusely so, above the base. The branching pattern is, in most cases, exceedingly irregular, and in some cases the branching pattern suggests that

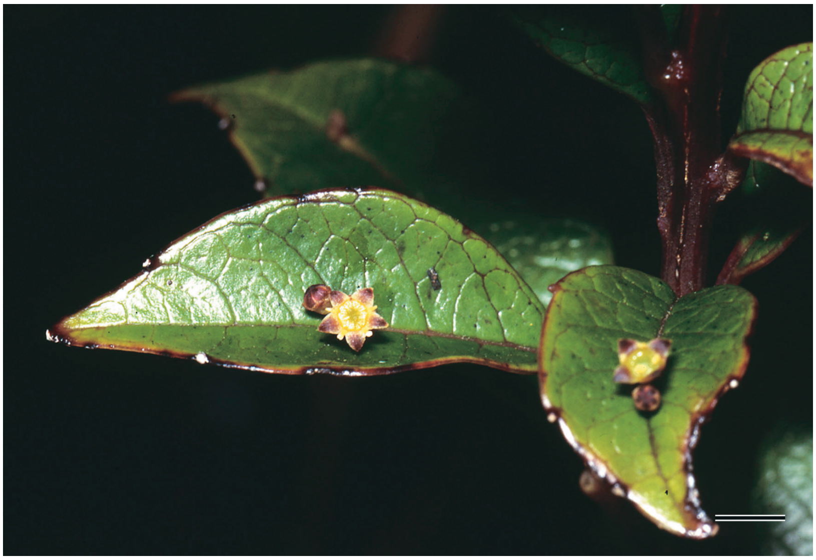

FIG. 1. Inflorescence of *Phyllonoma tenuidens*. Note its epiphyllous nature. Scale bar = 2mm. Photo by William A. Haber.

the inflorescence may be derived from a dichasmic cyme in which one side is aborted as noted by Rusby (1905) and Stork (1956). In contrast, in *Phyllonoma tenuidens*, *P. laticuspis*, and *P. weberbaueri*, the inflorescence appears to be a cluster of one to four unbranched racemes, and the flowers arise one above the other and are subtended by small, rather broad bracts which enclose the base of the pedicels and almost overlap one another.

Currently the inflorescences of *Phyllonoma* (Phyllonomaceae) are variously described. For instance, Stevens (2001 onwards) describes the inflorescences as "racemose, usually branched," and Every (2009) writes that they are "cymose and branched or racemose and unbranched fascicles or false umbels." Here I report on an analysis of the inflorescence structure of *Phyllonoma* based on anatomical observations of inflorescences of *P. tenuidens*. Like those of *P. weberbaueri* and *P. laticuspis*, the inflorescences of *P. tenuidens* are reduced in size, bearing several flowers and bracts condensed at the base (Fig. 1). Such a reduced inflorescence makes it difficult to understand the inflorescence structure under stereoscope. Only anatomical observations using microtome sections of the inflorescences can clarify the exact structure.

Materials and methods

Inflorescences of *Phyllonoma tenuidens*, which were collected from Provincia Puntarenas, Costa Rica (voucher: *William A. Haber 12765*, MO), were investigated. They were fixed and preserved in FAA (5 parts stock formalin, 5 parts glacial acetic acid, 90 parts 70% ethanol). Among them, three inflorescences were selected for anatomical observations. Externally, one of them appeared to consist of a single small flower bud (Fig. 2A); the two others appeared to consist of two open flowers and one or two small flower buds (Fig. 3A).

The three selected inflorescences were dehydrated through an ethanol series, then embedded in Technovit 7100 (Kulzer, Wehrheim, Germany) for microtoming. Serial resin sections cut at a thickness of 5μm were stained with Heidenhain's hematoxylin and mounted in Entellan.

Results

The inflorescences of *Phyllonoma tenuidens* are borne as a cluster of flowers on the adaxial

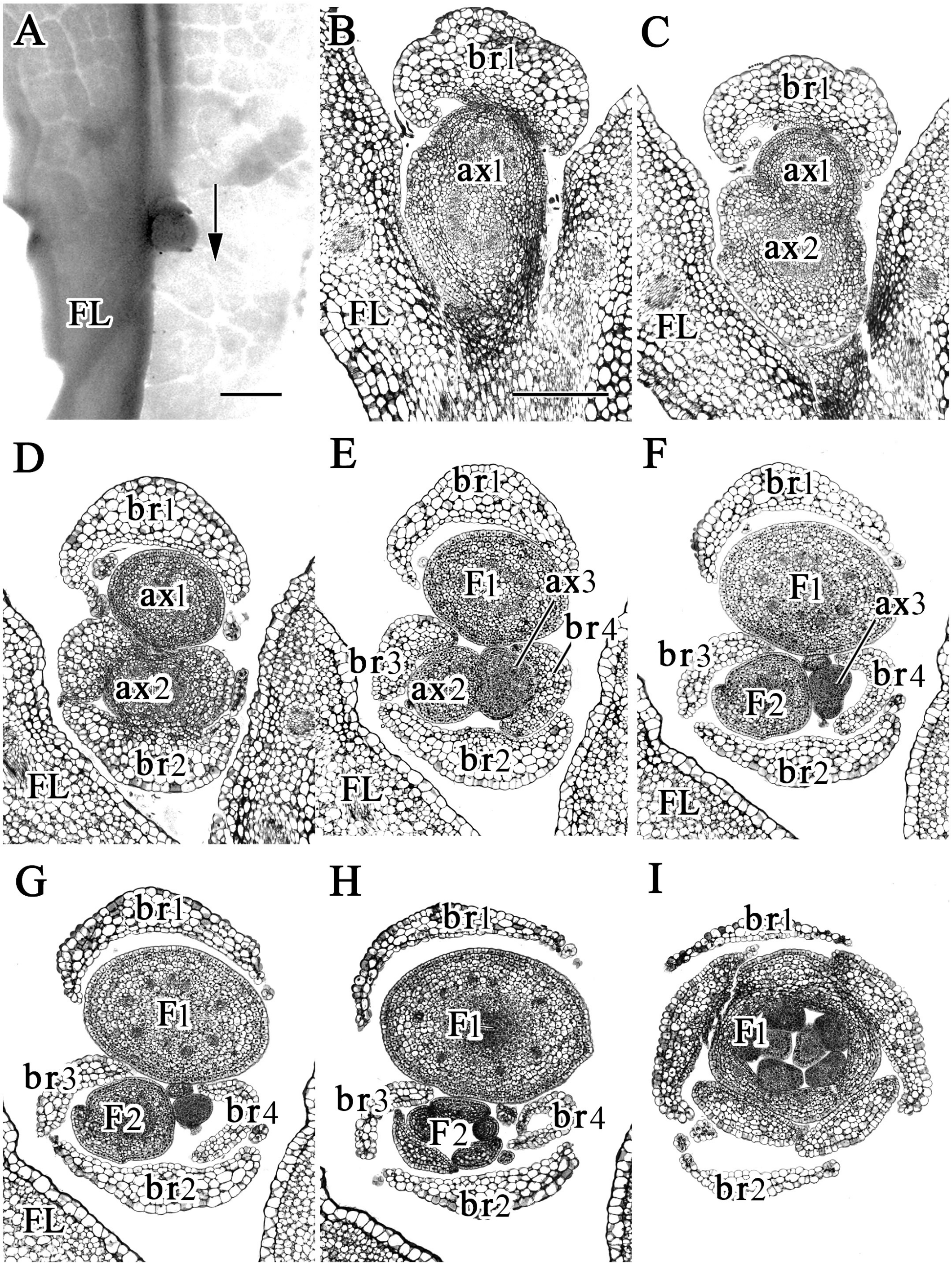

FIG. 2. Anatomy of inflorescence of *Phyllonoma tenuidens*. A: Inflorescence examined, which appears to consist of a single flower. Arrow shows the direction observed using transverse sections. B-I: Selected serial transverse sections of the basal part of the inflorescence presented in A. Note that only one of the two bracts subtends lateral axis, and that subtending bracts shift onto higher order axes. ax1, first order (primary) axis of the inflorescence examined; ax2 and ax3, second- and third order axis (lateral axis), respectively; br1 and br2, bracts on the first order axis; br3 and br4, bracts on the second order axis; F1 and F2, terminal flower of the first order and second order axis, respectively; FL, inflorescence-bearing foliage leaf. Scale bars = 700μm in A, 200μm in B, the scale in B also applies to C−I.

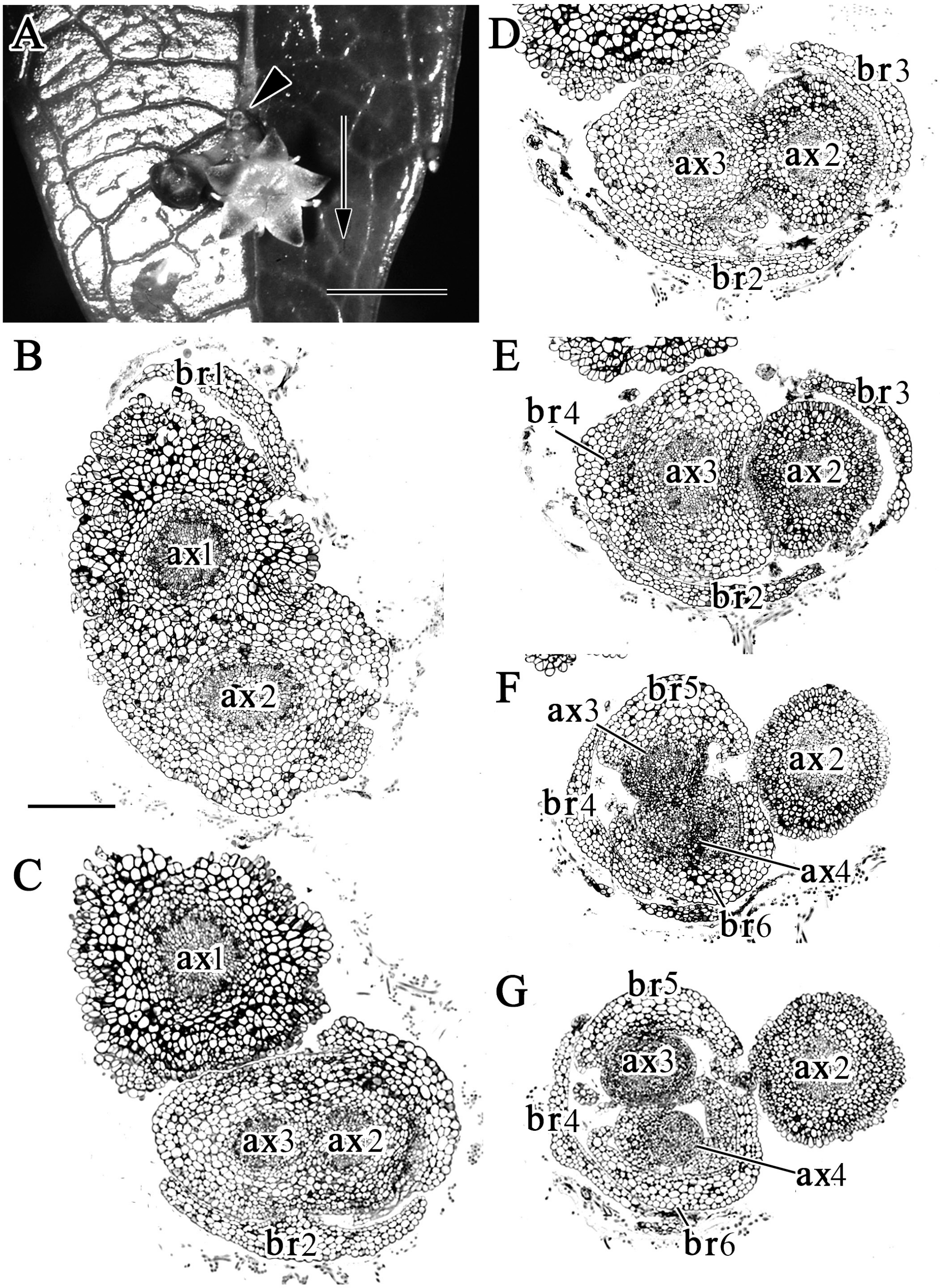

FIG. 3-1. Anatomy of inflorescence of *Phyllonoma tenuidens* with several developing flowers, showing a further ramification than in Fig. 2. A: Inflorescence examined. Arrowhead indicates a scar left by a fallen flower which corresponds to the primary axis. Arrow shows the direction observed using transverse sections. B-G: Selected serial transverse sections of the basal part of the inflorescence presented in A. Note that only one of the two bracts subtends lateral axis, and that subtending bracts always shift onto higher order axes. ax1, first order (primary) axis of the inflorescence examined; ax2 and ax3, second- and third order axis (lateral axis), respectively; br1 and br2, bracts on the first order axis; br3 and br4, bracts on the second order axis; br5 and br6, bracts on the third order axis. Scale bars = 2mm in A, 200μm in B; the scale in B also applies to C-G.

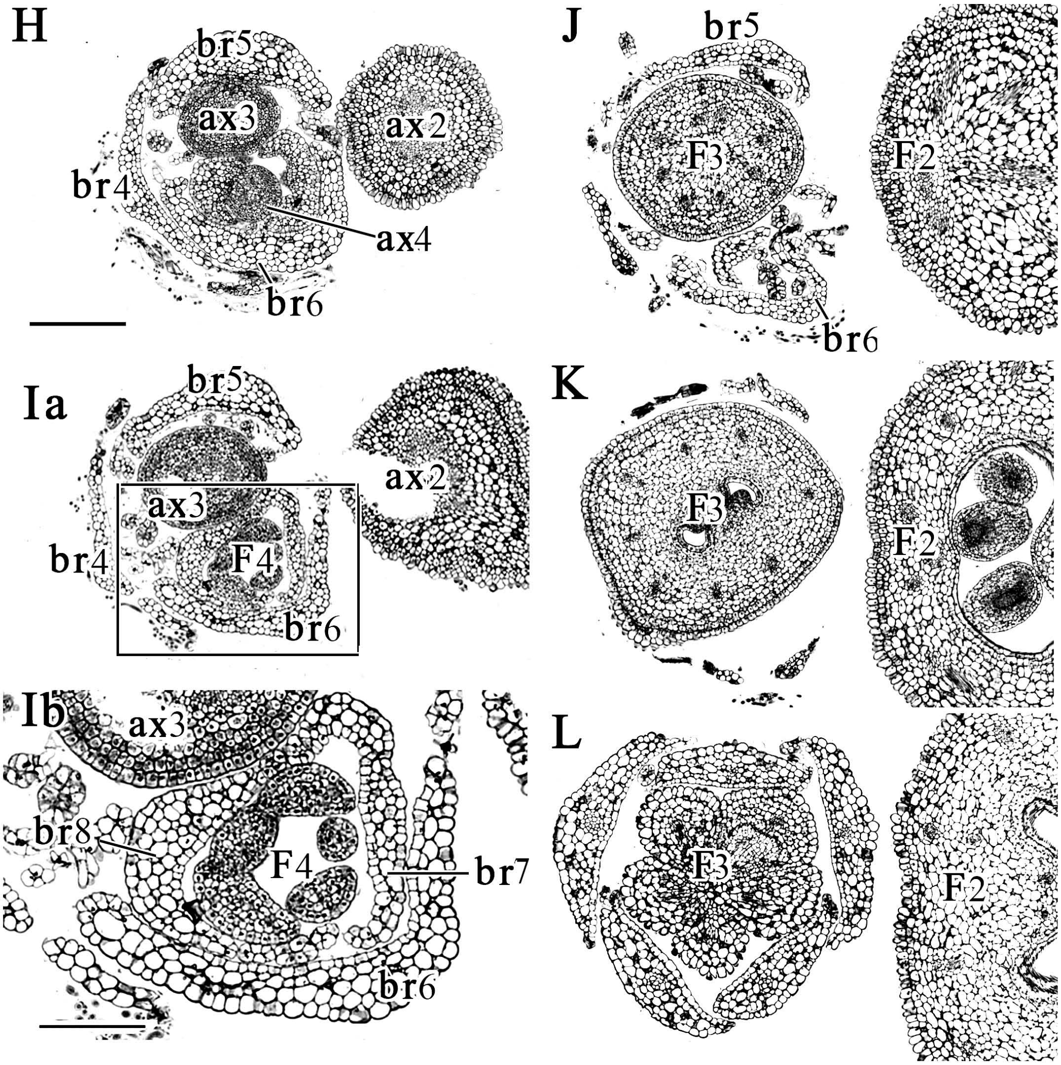

FIG.3-2. Continued. H-L: Selected serial transverse sections of the basal part of the inflorescence presented in A. Ib: Magnified view of the transverse section of the terminal flower (or the fourth order axis) enclosed by a rectangle in Ia, demonstrating the presence of two bracts in transversal regions. ax2, ax3, and ax4, second-, third-, and fourth order axis (lateral axis), respectively; br4, one of the two bracts on the second order axis; br5 and br6, bracts on the third order axis; br7 and br8, bracts on the fourth order axis; F2, F3, and F4, terminal flower of the second-, third-, and fourth order axis, respectively. The scale in B also applies to H-L.

surface of a foliage leaf (Fig. 1). Flowers in the same inflorescence greatly differ in developmental stage. I first observed the young inflorescence attached to the foliage leaf. From such observations, it is possible to determine where and how the inflorescence axes and bracts are positioned in relation to the inflorescence-bearing foliage leaf as well as to the leaf-bearing shoot. Subsequently, the two older inflorescences were observed to determine their mature structure.

Inflorescence with a single flower bud

The inflorescence-bearing foliage leaf is more or less conduplicate (Figs. 1, 2A), and V-shaped in transverse section (Fig. 2B-G). The inflorescence develops parallel to the midvein of the leaf and in an abaxial direction from the middle part of the foliage leaf (Fig. 2A). Transverse sections of the inflorescence-bearing foliage leaf thus provide transverse sections of the inflorescence simultaneously (Fig. 2B-I). When traced upwardly from the base using such transverse sections, the first order (primary) axis (ax1), which is short and terminated by a small flower bud (F1) (Fig. 2E-I), has the second order axis (ax2) on the abaxial side (Fig. 2B-D). The first order axis bears two bracts (prophylls): one (br1) on the adaxial side, and the other (br2) on the abaxial side (Fig. 2B-I). The former has no axillary bud, while the latter has the second order axis (ax2) in its axil (Fig. 2B-D). Noticeably, the bract (br2) subtending the second order axis (ax2) is positioned at a much higher level than the level at which the second order axis branches. That is, the bract subtending the second order axis is positioned on the second order axis itself, rather than on the first order axis (Fig. 2D).

The second order axis (ax2) is terminated by a smaller flower bud (F2) (Fig. 2F-H). Like the first order axis, it bears two bracts: one (br3) on the left side, and the other (br4) on the right side in transverse positions (Fig. 2E-H). The former lacks an axillary bud, while the latter produces the third order axis (ax3) from its axil (Fig. 2E). Here again, the bract (br4) subtending the third order axis is positioned on the third order axis itself (ax3), rather than on the second order axis (ax2) (Fig. 2E). The third order axis (ax3) terminates with a much smaller flower bud, and appears to have a fourth order axis (Fig. 2F, G). It is uncertain, however, in which direction the fourth order axis branches.

As observed above, the second-, third-, and probably fourth order axis successively branches from the first-, second-, and third order axis, respectively. The second order axis branches in an abaxial direction, and the third order axis in the transversal direction. Every higher order axis, like the first order axis, terminates in a single flower. Although the inflorescence examined appeared to have a single small flower bud, it actually bore at least two more flower buds. The question arises as to whether or not this pattern of branching proceeds as the inflorescence develops further? This will be better confirmed in subsequent analyses using the two additional inflorescences with several developing flowers.

Inflorescences with several developing flowers

One of the two inflorescences examined is illustrated in Fig. 3A. It was traced upwardly from the base to the apex using transverse sections of the inflorescence as in the inflorescence presented in Fig. 2A-I. The first order axis (ax1), which lost its terminal flower (see arrowhead in Fig. 3A), has a second order axis (ax2) that branches toward the abaxial side (Fig. 3B). The first order axis bears two bracts (prophylls), one (br1) on the adaxial side (Fig. 3B) and the other (br2) on the abaxial side (Fig. 3C). The former lacks an axillary bud while the latter has a second order axis in its axil. As in the inflorescence in Fig. 2A-I, the bract (br2) subtending the second order axis (ax2) is positioned on the second order axis itself, rather than on the first order axis (ax1) (Fig. 3B, C). The second order axis is terminated by a flower (F2) (Fig. 3C-L).

At a level slightly above the branching of the second order axis (ax2), the third order axis (ax3) branches toward the left side in a transverse position (Fig. 3C-E). Therefore, the third order axis in this inflorescence differs in its position from the third order axis in the inflorescence in Fig. 2A-I, which branched toward the right side. The second order axis also has two bracts (prophylls), one (br3) on the right side (Fig. 3D), and the other (br4) on the left side (Fig. 3E). The former lacks an axillary bud, while the latter has the third order axis in its axil. As in the inflorescence in Fig. 2A-I, the bract (br4) subtending the third order axis is positioned on the third order axis itself, rather than on the second order axis (Fig. 3E). The third order axis is terminated by a flower (F3) (Fig. 3J-L). The axis has two bracts, one adaxial and one abaxial (prophylls) (br5, br6) in reference to the main axis (Fig. 3F-H, Ia, J). Like the two bracts (br1, br3) of the first- and second order axes, the adaxial bract (br5) has no axillary bud. In contrast, like the bracts (br2, br4) of the first- and second order axes, the abaxial bract (br6) has a fourth order axis (ax4) in its axil. It is positioned on the fourth order axis rather than on the third order axis (Fig. 3F).

In this inflorescence, the fourth order axis has two bracts (prophylls) (br7, br8) in transversal positions (Fig. 3Ib). The presence of those bracts suggests that a fifth order axis can be formed in an axil of one (br8) of the two bracts, although it is not clearly recognizable. The fourth order axis terminates in a flower (F4) (Fig. 3Ia, Ib).

The third inflorescence examined had a similar branching pattern. Anatomical observations, however, showed that the third order axis branches from the second order axis toward the right rather than toward the left in the transverse position (Fig. 4A-C) as in the inflorescence in Fig. 2D and E.

Discussion

Inflorescence structure and bracts (prophylls) in Phyllonoma tenuidens

As documented above, *Phyllonoma tenuidens* has cymose branching. The inflorescence is not racemose as treated by Mori & Kallunki (1977) but a monochasial cyme, or more strictly a scorpioid cyme (or cincinnus). The first order axis has two bracts (prophylls), forming the second order axis from the bract axil on the abaxial side. The so-called recaulescence (for terminology see Jackson 1928) occurs between the subtending bract and the lateral axis. The adnation of the subtending bract to the lateral axis for some distance results in shifting of the bract to a higher order axis (Fig. 5A). Mori & Kallunki (1977) previously noted that "the inflorescence bracts and flowers are very often not closely associated", and that "in many cases, a flower has no subtending bract or a bract has no flower in its axil." Probably this pattern of metatopic displacement of organs (i.e., recaulescence) is prevalent throughout *Phyllonoma*. Weberling (1989) interpreted the epiphyllous inflorescence to be the result of recaulescence, although Stork (1956) and Dickinson & Sattler (1977; see also references cited therein) consider the inflorescence to be "truly phyllogenous," or produced from the leaf meristem. Weber (2004) showed that the inflorescence meristem developmentally shifted from the leaf–axil to an epiphyllous position in *P. laticuspis*.

The lateral axis also repeats ramification upwardly similar to the first order axis. All axes terminate in a flower. When the four-flowered inflorescence is viewed from the side, the inflorescence structure with regard to the first order and

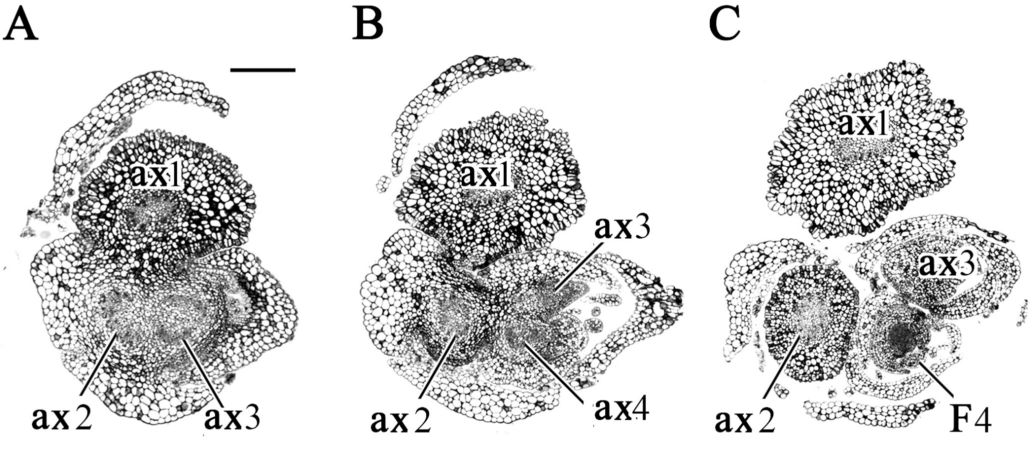

FIG.4. Inflorescence of *Phyllonoma tenuidens*, showing opposite direction of ramification in comparison to Fig. 3. A-C: Three selected serial transverse sections of the basal part of the inflorescence. ax1, ax2, ax3, and ax4, first, second-, third-, and fourth order axis (lateral axis), respectively; F4, terminal flower of the fourth order axis. Scale bar = 200μm in A, the scale in A also applies to B and C.

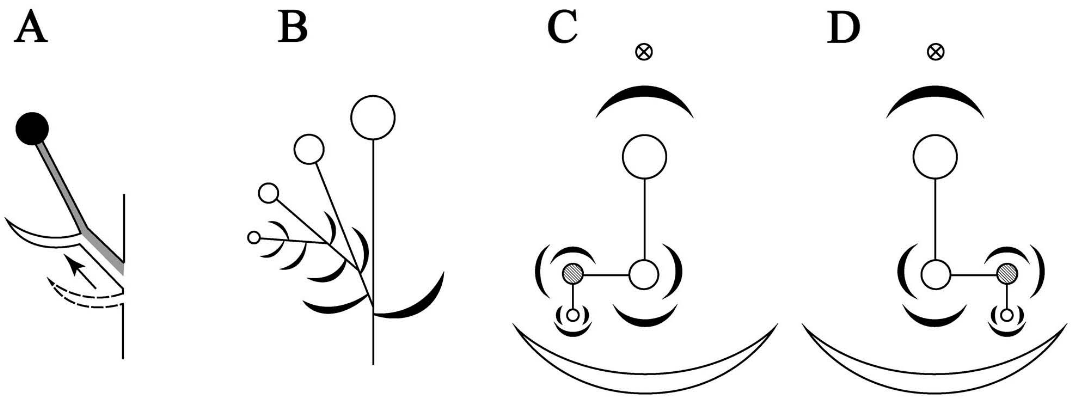

FIG.5. Diagrams illustrating the structure of monochasial cymes of *Phyllonoma*. A: Metatopic displacement of organs (i.e., recaulescence), which is likely common to the entire *Phyllonoma*. Note the shifting of a subtending bract onto a lateral axis as in *P. tenuidens*. A black circle shows an apical meristem of the lateral axis that has a potential to ramify further. B: Structure of a four–flowered inflorescence of *Phyllonoma tenuidens* viewed from lateral side. C and D: Structure of a four–flowered inflorescence of *Phyllonoma tenuidens* viewed from the top. Note that the third order axis (shaded circle) is formed at the left hand (C) and right-hand (D) bract axil of the second order axis. Flowers are indicated by white circles, and their sizes are shown smaller as they develop later.

three higher order axes and associated bracts (prophylls) can be presented as in Fig. 5B. Furthermore, the two diagrams in Fig. 5C and D illustrate the inflorescence structure of *Phyllonoma tenuidens* in relation to the subtending foliage leaf of the inflorescence when viewed from above. The difference between the two diagrams is in the third order axis forming on the left (Fig. 5C) or right side bract (prophyll) axil (Fig. 5D) of the second order axis. Of the three inflorescences examined in this study, two (Figs. 2, 4) represent the former, and one (Fig. 3) the latter.

General inflorescence structure in Phyllonoma

Now that it is clear that the reduced inflorescence of *Phyllonoma tenuidens* is cymose and in agreement with the inflorescence structure of *P. ruscifolia* (i.e., irregular monochasial cymes, see Mori & Kallunki 1977), then the reduced inflorescences of the other two species, *P. laticuspis* and *P. weberbaueri*, are also likely cymes, rather than racemes. Rusby (1905, p.191) reported that in *P. laticuspis* “a single flower first appears, followed by a branch on either side which develops into a very short pseudo–raceme, its rachis at length becoming peduncle-like.” This is a description of a monochasial cyme. Thus, although the inflorescences of the remaining species, *P. weberbaueri*, was unavailable for observation, inflorescences of the entire genus are likely monochasial, and probably scorpioid cymes.

Based on an analysis of inflorescences in *Phyllonoma*, Mori & Kallunki (1977) discussed a possible sequence of evolution from dichasial (not extant) to monochasial cymes. This evolutionary trend is not unusual in other angiosperms (Endress 2010). Although axillary buds are always lacking for the lateral axis, the presence of two bracts (prophylls) on every axis throughout the inflorescence of *P. tenuidens* suggests that the monochasial cymes of *Phyllonoma* were derived from dichasial cymes. Comparisons with inflorescences in related families of Aquifoliales, particularly in Helwingiaceae, which are sister to Phyllonomaceae, are needed to confirm this evolutionary scenario.

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